Recombinant Human Turbo Dicer Enzyme Kit

Genlantis
A division of Gene Therapy Systems, Inc.

PRODUCT SUMMARY

Cat. No: T520002 (50 Units)

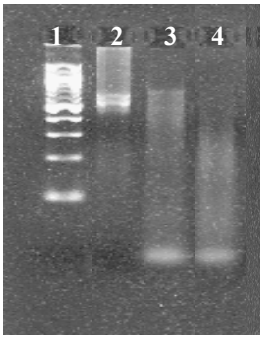
Kit Components

Description	Content
Recombinant Turbo Dicer Enzyme (0.5 unit/μl)	1 tube x 100 μl.
Dicer Reaction Buffer	1 tube x 50 μl.
50 mM $MgCl_2$ Solution	1 tube x 100 μl.
10 mM ATP	1 tube x 50 μl.
Dicer Stop Solution	1 tube x 100 μl.
5X BSA	1 tube x 50 μl
Nuclease-free Water	1 tube x 1 ml.

Shipping and Storage: The Recombinant Human Turbo Dicer Enzyme Kit is shipped frozen. For maximum stability and long-term use, immediately store at -20°C upon receipt. All components are stable for six months when stored properly.

Description: The Recombinant Human Turbo Dicer Enzyme Kit uses an ultra-active form of human recombinant dicer enzyme that can cleave more than 95% of dsRNA template into 22 bp siRNAs within 2 hours under optimized reaction conditions (Figure 1). This provides sufficient siRNAs in every single species to achieve effective gene silencing.

Fig. 1: Efficient Digestion of Double-Stranded RNA with Recombinant Human Turbo Dicer Enzyme



3% Agarose Gel

Lane#	Sample
1.	100 bp marker;
2.	1μg, 700 bp dsRNA
3.	1μg, 700 bp dsRNA, 15 hour digestion with 1 unit Dicer Enzyme
4.	1μg, 700 bp dsRNA, 2 hour digestion by 1 unit Turbo Dicer Enzyme.

NOTE: The Recombinant Human Dicer Enzyme Kit is intended FOR RESEARCH USE ONLY.

METHODS AND PROCEDURES

Preliminary Notes

1. We recommend that you use dsRNA templates of 500 – 1000 bp in length. The dicer enzyme might not digest well if the dsRNA is smaller than 300 bp.

2. For optimal results, we recommend using the GTS TurboScript™ T7 RNA Transcription Kit (Catalog# T510003) to generate double-stranded RNA (dsRNA); dsRNAs generated with TurboScript can be used directly with the Recombinant Turbo Dicer Enzyme Kit without purification, however dsRNAs purified using standard lithium chloride or equivalent procedures can give slightly better results.

Generation of siRNA Using Recombinant Turbo Dicer Enzyme

1. *Keep the Dicer Reaction Buffer at room temperature while assembling the reaction*. The following amounts are for a single 10 μl reaction.

3 μl – x	Nuclease-free water
x μl	dsRNA (1 μg)
1 μl	10 mM ATP
1 μl	5X BSA
2 μl	50 mM $MgCl_2$
1 μl	10X Dicer Reaction Buffer
2 μl	Recombinant Turbo Dicer Enzyme (0.5 unit/μl)

NOTE: *1 μg of dsRNA control template will yield about 0.5 μg of diced siRNA (d-siRNA) after purification with GTS' RNA Purification Columns 1 & 2; this is sufficient for one or two transfections in 24-well plates using the GeneSilencer siRNA Transfection Reagent. For more d-siRNA's, scale up the reaction volume proportionally.*

IMPORTANT *Avoid using excess Recombinant Dicer Enzyme, as it may decrease the amount of d-siRNA that can be generated from the digestion reaction.*

2. Incubate for 2 hours at 37°C.

3. Stop the reaction by adding 2 μl Dicer Stop Solution.

4. Check d-siRNA (~22 base pairs) by using either of the following two methods:
   a. 3% agarose Gel (TAE)
   b. 15% native polyacrylamide gel (29:1, cast in 1X and electrophoresed in 0.5X TBE). Run at 10 Watts and 4°C. Visualize RNA by staining with ethidium bromide

TROUBLESHOOTING GUIDE

Problem	Possible Causes	Recommended Solutions
d-siRNA yield is low.	No dsRNA or poor dsRNA quality.	Check the quality of the dsRNA produced in vitro. For best results, use the TurboScript™ T7 Transcription kit (Cat.# T510003). Also, check the amount of dsRNA added. If purification columns were used, make sure that the correct fractions were used; if columns or other purification method were not used, purify dsRNA before the dicer reaction.
	Too much Recombinant Dicer Enzyme was added.	Use 1 unit of the enzyme for every microgram of dsRNA.
	10 mM ATP is old.	Use fresh ATP solution.
Low silencing effect	Not enough d-siRNAs used	Check the amount of d-siRNA produced by gel electrophoresis.
	d-siRNA quality is poor	Purify the d-siRNAs; for best results, we recommend using the RNA purification Columns 1 and 2 (Cat# T510004 and T510005).

RELATED PRODUCTS

Product	Size	Cat. No.
For efficient generation of siRNA		
Dicer siRNA Generation Kit	5 genes / 50 transfections	T510001
TurboScript™ T7 Transcription Kit	20 Reactions	T510003
RNA Purification Column 1	20 Columns	T510004
RNA Purification Column 2	20 Columns	T510005
For efficient and functional siRNA transfection		
GeneSilencer® siRNA Transfection Reagent	200 reactions (0.75 ml)	T500750
GeneSilencer® siRNA Transfection Reagent	5 x 200 reactions (5 x 0.75 ml)	T505750
For efficient transfection of DNA into diverse cell lines		
GenePORTER® 2 Transfection Reagent	75 reactions (0.75 ml)	T202007
GenePORTER® 2 Transfection Reagent	150 reactions (1.5 ml)	T202015
GenePORTER® 2 Transfection Reagent	750 reactions (5 x 1.5 ml)	T202075
For 3-minute transformation into E. coli		
TurboCells® Chemically Competent *E. coli*	20 x 50 µl	C300020
TurboCells® F′ Chemically Competent *E. coli*	20 x 50 µl	C301020

NOTE: The Recombinant Human Dicer Enzyme Kit is intended FOR RESEARCH USE ONLY.

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